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Title: Orthotopic Rat Forelimb Transplantation

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

No

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Leica M620

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

2.2.1, 2.2.2, 2.2.3, 2.2.4; 2.3.1; 2.4.1, 2.4.2, 2.4.3, 2.4.4, 2.4.5; 2.5.1; 2.6.1; 2.7.1, 2.7.2, 2.7.3, 2.7.4; 2.9.1, 2.9.2, 2.10.1, 2.10.2; 3.1.1, 3.1.2; 3.2.1, 3.2.2, 3.2.3; 3.3.1; 3.4.1; 3.5.1; 3.6.1

Video editor: The scope shots were filmed by the videographer.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 26

Number of Shots: 49

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Videographer notes: Only 1 Author was present, so they did all the interviews.

Notes: Asked the authors for the name of the author who gave the interview. Will update once they reply.

~~REQUIRED: What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Gerald Brandacher:** The research focuses on rat forelimb transplantation to study immune rejection, functional recovery, and potential strategies for tolerance induction.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer notes: Accidentally filmed 1.2 interview first and mislabeled 1.1

~~What are the current experimental challenges?~~

- 1.2. **Yinan Guo:** Current experimental challenges include establishing a stable model and ensuring reproducibility across procedures.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer notes: Accidentally filmed 1.2 interview first and mislabeled 1.1

~~What advantages does your protocol offer compared to other techniques?~~

- 1.3. **Byoung Chol Oh:** Our protocol is cost-effective and offers high reproducibility compared to other techniques.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Johns Hopkins University Animal Care and Use Committee according to guidelines established by the National Institutes of Health and the American Association for the Accreditation of Laboratory Animal Care

Protocol

2. Procedure for Harvesting Donor Forelimb Graft

Demonstrator: Byoung Chol Oh

2.1. To begin, make a circumferential incision approximately 1 centimeter above the elbow [1-TXT] and divide the skin to expose the superficial subcutaneous tissue without damaging it [2].

2.1.1. WIDE: Talent makes a circumferential incision 1 centimeter above the elbow.
TXT: Anesthesia; Induction: 5% Isoflurane; Maintenance: 1% Isoflurane

2.1.2. A shot of the exposed superficial subcutaneous tissue.

2.2. Using blunt dissection, separate the subcutaneous tissue and fascia [1], cauterize all superficial vessels [2], and transect the tissue [3]. Expose the deltoid, a portion of the pectoralis major, and the triceps brachii muscles [4].

2.2.1. SCOPE: Talent dissecting subcutaneous tissue and fascia with blunt instruments.
Videographer's notes: 2.2.1 – 2.2.4 are combined.

Videographer: Please film the SCOPE shots using the scope kits

2.2.2. SCOPE: Talent cauterizing the superficial vessels.

2.2.3. SCOPE: Talent transecting the tissue.

2.2.4. SCOPE: A shot of the exposed deltoid, a portion of the pectoralis major, and the triceps brachii muscles.

2.3. Then, bluntly dissect underneath the pectoralis major muscle to reveal the brachial vessels, median nerve, and ulnar nerve [1].

2.3.1. SCOPE: Talent bluntly dissecting under the pectoralis major muscle to expose brachial vessels and median and ulnar nerves.

2.4. Cauterize the superficial vein on the deltoid muscle [1] and transect it proximally [2]. Transect the deltoid muscle perpendicular to the muscle fiber orientation [3]. Then, dissect and transect the pectoralis major and minor muscles [4] to reveal the axillary artery, vein, and nerve [5].

2.4.1. SCOPE: Talent cauterizing the superficial vein on the deltoid muscle.
Videographer's notes: 2.4.1 – 2.4.5 are combined

2.4.2. SCOPE: Talent transecting the superficial vein proximally.

- 2.4.3. SCOPE: Talent cutting the deltoid muscle perpendicular to the muscle fiber orientation.
- 2.4.4. SCOPE: Talent dissecting and transecting the pectoralis major and minor muscles.
- 2.4.5. SCOPE: A shot of the exposed axillary artery, vein, and nerve.
- 2.5. Transect the median, ulnar, and radial nerves at their proximal ends, preserving as much length as possible [1].
 - 2.5.1. SCOPE: Talent carefully transecting the median, ulnar, and radial nerves while preserving maximum nerve length.
- 2.6. Dissect and free the brachial artery and vein [1].
 - 2.6.1. SCOPE: Talent dissecting and freeing the brachial artery and vein from surrounding tissues.
- 2.7. Transect the biceps brachii [1], triceps brachii [2], and latissimus dorsi muscles [3] to fully expose the humerus [4].
 - 2.7.1. SCOPE: Talent cutting the biceps brachii. Videographer's notes: 2.7.1 – 2.7.4 are combined
 - 2.7.2. SCOPE: Talent cutting the triceps brachii.
 - 2.7.3. SCOPE: Talent cutting the latissimus dorsi muscles.
 - 2.7.4. SCOPE: A shot of the exposed humerus.
- 2.8. Using rongeur forceps, transect the bone distal to the deltoid tuberosity of the humerus [1].
 - 2.8.1. Talent using rongeur forceps to cut the bone distal to the deltoid tuberosity of the humerus. Videographer's notes: Rolled both cameras (audio slate only due to camera position)
- 2.9. After gently inspecting the lumen of the brachial artery and vein [1], flush the graft with 6 milliliters of heparinized saline [2].
 - 2.9.1. SCOPE: Talent checking the lumen of the brachial artery and vein. Videographer's notes: 2.9.1 and 2.9.2 are combined
 - 2.9.2. SCOPE: Talent flushing the graft with a syringe containing heparinized saline.
- 2.10. Mount a polyimide cuff on the brachial artery [1] and vein, respectively [2].

2.10.1. SCOPE: Talent attaching a polyimide cuff to the brachial artery. Videographer's notes: 2.10.1 and 2.10.2 are combined

2.10.2. SCOPE: Talent attaching a polyimide cuff to the vein.

2.11. Store the graft wrapped in wet cotton gauze at 4 degrees Celsius [1].

2.11.1. Talent placing the graft wrapped in wet cotton gauze into a freezer. Videographer's notes: (audio slate only due to camera position)

2.12. After euthanizing the donor rat, perform a thoracotomy [1-TXT] and excise the heart [2].

2.12.1. Talent performing a thoracotomy. TXT: Euthanasia: Isoflurane overdose Videographer's notes: 2.12.1 and 2.12.2 are combined

2.12.2. Talent excising the heart.

3. Recipient Limb Reconstruction Procedure and Post-Transplant Management

3.1. After dissection and transection of the vessels and nerves as demonstrated for the donor rat, transect the humerus at the deltoid tuberosity [1-TXT] and seal the marrow cavity using bone wax [2].

3.1.1. SCOPE: Talent transecting the humerus at the deltoid tuberosity. TXT: Anesthesia; Induction: 5% Isoflurane; Maintenance: 1% Isoflurane Videographer's notes: 3.1.1 and 3.1.2 are combined

3.1.2. SCOPE: Talent applying bone wax to seal the marrow cavity. Videographer's notes: Per author this was filmed on SCOPE camera

3.2. Use an intramedullary rod to reconnect the bone marrow cavity of the donor and recipient [1]. Use bone wax to seal the connection [2]. Suture the triceps brachii muscle with a 6-0 (six-oh) absorbable suture to create a platform for vessel anastomosis [3].

3.2.1. SCOPE: Talent inserting an intramedullary rod to connect the donor and recipient bone marrow cavities. Videographer's notes: 3.2.1 - 3.2.3 are combined, 3.2.1 – 3.2.3 filmed on SCOPE camera (per Author)

3.2.2. SCOPE: Talent sealing the connection with bone wax.

3.2.3. SCOPE: Talent suturing the triceps brachii muscle with a 6-0 absorbable suture.

3.3. Perform epiperineurial neurorrhaphy with an interrupted 10-0 (ten-oh) suture of the radial nerve [1].

3.3.1. SCOPE: Talent performing epiperineurial neurorrhaphy with interrupted 10-0 suture of the radial nerve.

3.4. Then, perform the vascular anastomosis using a non-suture cuff technique [1].

3.4.1. SCOPE: Talent performing vascular anastomosis using a non-suture cuff technique. Videographer's notes: Filmed on SCOPE camera (per Author)

3.5. Again, perform epiperineurial neurorrhaphy with interrupted 10-0 sutures to reconnect the median and ulnar nerves [1].

3.5.1. SCOPE: Talent reconnecting the median and ulnar nerves using interrupted 10-0 epiperineurial sutures. Videographer's notes: 3.5.1 was filmed with 3.4.1 but not slated as such

3.6. Reconnect the deltoid and pectoralis major muscles using interrupted sutures [1].

3.6.1. SCOPE: Talent suturing the deltoid and pectoralis major muscles with interrupted sutures.

3.7. Now, close the wound with a 4-0 non-absorbable suture [1]. Administer warm, sterile saline subcutaneously at 2% of body weight at the end of surgery, observe the animal under a warming lamp for at least 4 hours, return it to the housing facility, and continue postoperative monitoring [2-TXT].

3.7.1. Talent closing the wound using 4-0 non-absorbable sutures.

3.7.2. Talent injecting warm, sterile saline subcutaneously at a volume equivalent to 2 percent of the body weight.

Videographer's notes: Author notified that they are unable to film sections 3.8, 3.9, 3.10, 3.11, 3.12, 3.13, 3.14, because the animal needs time to heal before these steps can be filmed.

NOTES: For removed steps, VO is added concisely in the previous step

~~3.8. Return the animal to the housing facility once it is alert, able to upright itself, demonstrates coordinated movement, and independently obtains water and food [1].~~

~~3.8.1. Talent placing the recovered animal back into the housing facility.~~

~~3.9. Monitor the animals daily for pain and distress, as outlined in the checklist [1].~~

~~3.9.1. TEXT on PLAIN BACKGROUND:~~

- ~~1. Check for anorexia (absence of feces in the cage).~~
- ~~2. Check for dehydration (skin tenting after pinching).~~
- ~~3. Check for hunched posture, immobility, or guarding of the incision site.~~
- ~~4. Check for failure to groom (ruffled or dirty coat).~~
- ~~5. Check for excessive licking, scratching, redness, swelling, or self mutilation.~~
- ~~6. Check for aggressive behavior when handled.~~
- ~~7. Check for panting, labored breathing, or reddish brown nasal/ocular discharge.~~
- ~~8. Check for signs of sepsis.~~

~~3.10. Remove the sutures at 14 days post-operation [1].~~

~~3.10.1. Talent using forceps and scissors to remove sutures from the incision site.~~

~~3.11. Administer a second dose of buprenorphineSR (*buprenorphine S-R*) subcutaneously at a dose of 1 milligram per kilogram on postoperative day 2 [1-TXT].~~

~~3.11.1. Talent injecting buprenorphineSR subcutaneously into the animal. **TXT: 48 h after the initial dose**~~

~~3.12. Under general anesthesia, fix the animal's forelimb in 90 degrees abduction [1]. Place two needle electrodes 1 millimeter apart in the axilla near the insertion of the pectoralis major onto the humerus [2].~~

~~3.12.1. Talent positioning the anesthetized animal's forelimb at 90 degrees abduction.~~

~~3.12.2. Talent inserting two needle electrodes spaced 1 millimeter apart into the axillary region.~~

~~3.13. Stimulate the proximal median nerve percutaneously at 10 volts to induce maximal tetanic contraction of the flexors [1].~~

~~3.13.1. Stimulation being applied to the median nerve at 10 volts and capturing muscle contraction.~~

~~3.14. Once full digital flexion forms a grasp [1], place a metal loop connected to a force meter on the palm [2] and secure it within the flexed digits [3]. Pull the force meter until the grasp is lost [4] and record the maximum force in Newtons [5].~~

~~3.14.1. A shot of the full digital flexion forming a grasp.~~

~~3.14.2. Talent placing a metal loop connected to a force meter on the palm.~~

~~3.14.3. Talent securing the metal loop with the flexed digits.~~

~~3.14.4. Talent pulling the force meter gradually until the grasp is lost.~~

~~3.14.5. Talent recording the force reading.~~

Results

4. Results

4.1. Graft viability was assessed over time using a survival curve to monitor recipient outcomes following forelimb transplantation [1]. All animals that received a forelimb transplant survived through the 120-day observation period, indicating complete graft survival and procedural success [2].

4.1.1. LAB MEDIA: Figure 4.

4.1.2. LAB MEDIA: Figure 4. *Video Editor: Highlight the horizontal straight line with three dots at 100.*

4.2. To further assess graft integrity, histological examination of the skin was conducted at the study endpoint, revealing preserved epidermal and dermal architecture along with intact appendages, which suggested healthy skin regeneration in the graft [1].

4.2.1. LAB MEDIA: Figure 5.

4.3. Similarly, histological analysis of the grafted muscle showed aligned fibers and evenly spaced nuclei, indicating preserved muscle structure [1].

4.3.1. LAB MEDIA: Figure 6. *Video Editor: Highlight the blue points when the VO says, "evenly spaced nuclei".*

4.4. Functional recovery was quantified using a grip strength test performed over 21 weeks post-transplantation [1], showing a steady increase in grip force [2] that plateaued after 15 weeks [3], suggesting effective reinnervation and restoration of limb function [4].

4.4.1. LAB MEDIA: Figure 8.

4.4.2. LAB MEDIA: Figure 8. *Video Editor: Highlight the portion of the plot from 4 to 15 to show a steady increase of the curve.*

4.4.3. LAB MEDIA: Figure 8. *Video Editor: Highlight the portion of the plot from 15 to end to show that the curve has plateaued.*

4.4.4. LAB MEDIA: Figure 8.

Pronunciation guides:

1. Circumferential
Pronunciation link: <https://www.merriam-webster.com/dictionary/circumferential>
IPA: /ˌsɜː.kəm.fəˈren(t)ʃəl/
Phonetic Spelling: sur·kuhm·fuh·ren·shuhl
2. Subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌb.kjuˈteɪ.ni.əs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
3. Dissection
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissection>
IPA: /dɪˈsek.ʃən/
Phonetic Spelling: dye·sek·shuhn
4. Fascia
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascia>
IPA: /ˈfæʃ.i.ə/, /ˈfeɪʃi.ə/
Phonetic Spelling: fash·ee·uh; fay·shee·uh
5. Cauterize
Pronunciation link: <https://www.merriam-webster.com/dictionary/cauterize>
IPA: /ˈkɔː.tə.raɪz/
Phonetic Spelling: kaw·tuh·ryze
6. Transect
Pronunciation link: <https://www.merriam-webster.com/dictionary/transect>
IPA: /trænˈsekt/
Phonetic Spelling: tran·sekt
7. Deltoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/deltoid>
IPA: /ˈdel.tɔɪd/
Phonetic Spelling: del·toyd
8. Pectoralis
Pronunciation link: <https://www.merriam-webster.com/dictionary/pectoralis>
IPA: /ˌpek.təˈræl.ɪs/
Phonetic Spelling: pek·tuh·ral·iss
9. Triceps
Pronunciation link: <https://www.merriam-webster.com/dictionary/triceps>
IPA: /ˈtraɪ.sɛps/
Phonetic Spelling: try·seps
10. Brachii
Pronunciation link: <https://www.merriam-webster.com/dictionary/brachii>
IPA: /ˈbreɪ.ki.aɪ/
Phonetic Spelling: bray·kee·eye

11. Brachial

Pronunciation link: <https://www.merriam-webster.com/dictionary/brachial>

IPA: /'breɪ.ki.əl/

Phonetic Spelling: bray·kee·uhl

12. Median

Pronunciation link: <https://www.merriam-webster.com/dictionary/median>

IPA: /'miː.di.ən/

Phonetic Spelling: mee·dee·uhn

13. Ulnar

Pronunciation link: <https://www.merriam-webster.com/dictionary/ulnar>

IPA: /'ʌl.nər/

Phonetic Spelling: ul·nur

14. Proximally

Pronunciation link: <https://www.merriam-webster.com/dictionary/proximally>

IPA: /'prɑːk.sə.mə.li/

Phonetic Spelling: prok·suh·muh·lee

15. Axillary

Pronunciation link: <https://www.merriam-webster.com/dictionary/axillary>

IPA: /'æk.sə.lər.i/

Phonetic Spelling: ak·suh·lair·ee

16. Radial

Pronunciation link: <https://www.merriam-webster.com/dictionary/radial>

IPA: /'reɪ.di.əl/

Phonetic Spelling: ray·dee·uhl

17. Latissimus

Pronunciation link: <https://www.merriam-webster.com/dictionary/latissimus>

IPA: /lə'tɪs.ə.məs/

Phonetic Spelling: luh·tiss·uh·muhs

18. Dorsi

Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsi>

IPA: /'dɔːr.səɪ/

Phonetic Spelling: dor·sye

19. Humerus

Pronunciation link: <https://www.merriam-webster.com/dictionary/humerus>

IPA: /'hjuː.mə.rəs/

Phonetic Spelling: hyoo·muh·ruhs

20. Rongeur

Pronunciation link: <https://www.merriam-webster.com/dictionary/rongeur>

IPA: /rɑːn'ʒɜː/

Phonetic Spelling: rahn·zhur

21. Tuberosity

Pronunciation link: <https://www.merriam-webster.com/dictionary/tuberosity>

IPA: /ˌtuː.bəˈrɑː.sə.ti/

Phonetic Spelling: too·buh·rah·suh·tee

22. Lumen

Pronunciation link: <https://www.merriam-webster.com/dictionary/lumen>

IPA: /ˈluː.mən/

Phonetic Spelling: loo·muhn

23. Heparinized

Pronunciation link: <https://www.merriam-webster.com/dictionary/heparin>

IPA: /ˈhep.ə.rɪn/

Phonetic Spelling: hep·uh·rin

24. Polyimide

Pronunciation link: <https://www.merriam-webster.com/dictionary/polyimide>

IPA: /ˌpɑː.liˈɪm.aɪd/

Phonetic Spelling: pol·ee·im·ide

25. Gauze

Pronunciation link: <https://www.merriam-webster.com/dictionary/gauze>

IPA: /ɡɔːz/

Phonetic Spelling: gawz

26. Euthanizing

Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanize>

IPA: /ˈjuː.θəˌnaɪz/

Phonetic Spelling: yoo·thuh·nyze

27. Thoracotomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/thoracotomy>

IPA: /ˌθɔːr.əˈkɑː.tə.mi/

Phonetic Spelling: thor·uh·kot·uh·mee

28. Excise

Pronunciation link: <https://www.merriam-webster.com/dictionary/excise>

IPA: /ɪkˈsaɪz/

Phonetic Spelling: ik·syze

29. Intramedullary

Pronunciation link: <https://www.merriam-webster.com/dictionary/intramedullary>

IPA: /ˌɪn.trəˈmed.jə.lər.i/

Phonetic Spelling: in·truh·med·yuh·lair·ee

30. Absorbable

Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbable>

IPA: /əbˈzɔːr.bə.bəl/

Phonetic Spelling: ub·zor·buh·buhl

31. Anastomosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/anastomosis>

IPA: /əˌnæs.təˈmoʊ.sɪs/

Phonetic Spelling: uh·nas·tuh·moh·suhs

- 32. Epiperineurial
Pronunciation link: No confirmed link found
IPA: /ˌep.i.pɪr.ə.nəʔri:.əl/
Phonetic Spelling: ep·ee·per·uh·noor·ee·uhl
- 33. Neurorrhaphy
Pronunciation link: <https://www.merriam-webster.com/dictionary/neurorrhaphy>
IPA: /nəʔrɔ:r.ə.fi/
Phonetic Spelling: noor·or·uh·fee
- 34. Vascular
Pronunciation link: <https://www.merriam-webster.com/dictionary/vascular>
IPA: /ˈvæs.kjə.lə/
Phonetic Spelling: vas·kyuh·ler
- 35. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /ˌaɪ.souˈflɔr.eɪn/
Phonetic Spelling: eye·soh·floor·ayn
- 36. Saline
Pronunciation link: <https://www.merriam-webster.com/dictionary/saline>
IPA: /ˈseɪ.laɪn/
Phonetic Spelling: say·line